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Clinical validation of renin monoclonal antibody-based sandwich assays of renin and prorenin, and use of renin inhibitor to enhance prorenin immunoreactivity

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Newly developed IRMAs to measure the plasma concentrations of renin and prorenin were validated for clinical use and compared with a classical enzyme kinetic assay. The IRMAs involve two monoclonal antibodies, one that reacts equally well with renin and prorenin and one that recognizes renin well but prorenin only minimally. Prorenin reactivity with the second antibody was enhanced by adding the renin inhibitor, Remikiren, to plasma. The complex of prorenin with this active-site ligand undergoes a conformational change, whereby prorenin is converted into a form that cannot be differentiated from renin by the IRMA. The linear working range of the assay was 4.0–3000 mU/L. The concentration of prorenin was calculated by subtracting the assay result obtained without Remikiren (i.e., renin) from the result obtained with Remikiren (i.e., renin plus prorenin). No more than 2% of prorenin present in plasma was detected as renin. The interassay CVs for renin quantification were 18%, 13%, and 8% at low, medium, and high concentrations, respectively. The interassay CV for calculated prorenin was 8% at both low and high concentrations. The IRMA results were highly correlated with those of an enzyme kinetic assay in healthy subjects; in patients with such conditions as primary hyperaldosteronism, renovascular hypertension, and low-, medium-, and high-renin essential hypertension; and in women undergoing gonadotropin stimulation.

INDEXING TERMS: avidin–biotin interaction • immunoradiometric assay • reference values • renin inhibitor • Remikiren • hypertension • hyperaldosteronism • infertility studies • angiotensin • enzyme kinetic method compared

The aspartic protease renin, which is produced by the kidney, is essential for the regulation of a series of reactions leading to the formation of angiotensin (Ang) II.[4] Ang II is a key hormone in blood pressure regulation and in maintaining water and sodium homeostasis *[1]*. Recent studies indicate that Ang II may also play important roles in cardiac and vascular hypertrophy *[2, 3]* and in reproductive physiology *[4]*.

Because measurements of plasma Ang II concentrations are notoriously inaccurate unless a laborious HPLC step is included and special blood collection procedures are used, the in vivo activity of the renin–angiotensin system is usually assessed with the so-called plasma renin activity (PRA) assay *[5]*. In this assay plasma is incubated at 37 °C, without the addition of renin substrate (angiotensinogen), and the quantity of Ang I that is generated in vitro from endogenous angiotensinogen is measured by RIA. PRA measurements are used to differentiate between primary and secondary aldosteronism, to diagnose renovascular hypertension, and to monitor mineralocorticoid supplementation therapy *[6]*. PRA assays are also performed to

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[4] Nonstandard abbreviations: Ang, angiotensin; ACE, angiotensin-converting enzyme; hFSH, human follicle-stimulating hormone; hLH, human luteinizing hormone; IRP, International Reference Preparation; PBS, phosphate-buffered saline; PRA, plasma renin activity; PRC, plasma renin concentration.

Table 1. Properties of monoclonal renin antibodies.

Antibody code	Specificity	Ab conc for 50% binding of ^{125}I-renin, 10^{-9} mol/L	Inhibition of Ang I-generating activity, 10^{-9} mol/L	Type of inhibition	Ref.
R 3-36-16	Renin and prorenin	0.062	IC_{50} = 0.013	Noncompetitive	*23, 24*
R 1-20-5	Renin	250	IC_{50} = 14	Competitive	*24*
F 55-3E8	Renin and prorenin	1.0	No inhibition		*28, 29*
F 55-4G1	Renin	0.35	K_i = 0.25–0.40	Noncompetitive	*28, 29*

IC_{50}, concentration that inhibits 50% of activity.

divide patients with essential hypertension into low-, medium-, and high-renin categories, which can help in selecting the optimal therapeutic approach *[7]*.

In vivo, the decapeptide Ang I is converted to Ang II by the zinc-containing angiotensin-converting enzyme (ACE). In the PRA assay sufficient EDTA is added to the plasma to inactivate ACE, and thereby block the conversion of Ang I to Ang II. Results of the PRA assay must be interpreted with some caution. A single fixed incubation time is used in routine assays, during which period it is assumed that the generation of Ang I is linear. Because the concentration of angiotensinogen in normal plasma is close to the Michaelis constant, K_M, the measured PRA depends not only on the concentration of renin but also on that of angiotensinogen *[8]*. PRA is therefore not always a good measure of the release of renin by the kidneys into the circulation.

By adding saturating amounts of homologous or heterologous angiotensinogen to plasma, the rate of Ang I formation by renin becomes independent of the concentration of renin substrate. This is the principle of the plasma renin concentration (PRC) assay. Our in-house PRC assay uses a heterologous substrate, sheep angiotensinogen, as the Ang I precursor, which affords the advantages of a lower K_M and a higher maximal reaction rate, V_{max}, than with homologous substrate *[8]*.

Plasma contains both renin and its precursor, prorenin, which is inactive in enzyme kinetic assays. In contrast with renin, prorenin is released into the circulation not only by the kidney but also by other organs, i.e., ovary *[9]*, testis *[10]*, and possibly the adrenal gland *[11]* and the uteroplacental unit *[12]*. In normal plasma the concentration of prorenin is about ninefold that of renin. The release of renin from the kidney is closely controlled (regulated secretion), but the release of prorenin from the kidney and other organs is not (constitutive secretion). The secretion of renin responds rapidly to stimulation, but prorenin concentrations increase only after prolonged stimulation *[13]*. For clinical purposes, prorenin is assayed to detect renin-producing tumors such as nephroblastoma in children *[14, 15]*, and to identify patients with diabetes mellitus at risk of microvascular complications *[16, 17]*. Plasma prorenin can also be used as an index of ovarian response to stimulation by administered gonadotropin *[9]*.

Prorenin is usually measured with the above-mentioned enzyme kinetic assays after trypsin has been added to the samples to convert prorenin to renin by cleaving the N-terminal prosegment *[5, 13]*. A problem with the use of trypsin as a first step in prorenin assays is the possibility that prorenin and renin may be proteolytically destroyed, resulting in a loss of enzymatic activity. Prorenin can also be activated nonproteolytically by exposing plasma to low pH (acid-activation) or low temperature (cryoactivation) *[18, 19]*. These procedures, however, are generally not accepted as being useful for clinical prorenin assays because the activation is reversible and often incomplete.

Monoclonal antibodies (MAbs) that react with both renin and prorenin, and monoclonal antibodies that are specific for renin, have recently become available. The three-dimensional structure of renin is bilobal, the active site being located within a deep cleft between the lobes *[20]*. Native prorenin is enzymatically inactive because the cleft is occupied by the prosegment. Low-molecular-mass active-site ligands can enter the cleft and induce a slow conformational change of the inactive (closed) form of the prorenin molecule, through which the molecule can attain the active (open) form, with the active site exposed *[21]*. To induce this conformational change, we used the specific renin inhibitor, Remikiren *[22]*. The open form of prorenin can be detected with a monoclonal renin antibody that recognizes renin but not the closed form of prorenin.

On the basis of these findings we developed novel IRMAs to measure the plasma concentrations of renin and prorenin. The concentration of prorenin was calculated by subtracting the assay result obtained without Remikiren (i.e., renin) from the result obtained with Remikiren (i.e., renin plus prorenin). Here we report on the validation of these assays for clinical use. We also compared the performances of these assays with our in-house enzyme kinetic PRC assay.

Materials and Methods

MATERIALS

Renin MAbs. Table 1 provides information on the properties of the MAbs we used. MAb R 3-36-16 and MAb R 1-20-5 were from Nichols Institute Diagnostics (Wychen, The Netherlands). MAb 3-36-16 reacts equally well with prorenin as with active renin, whereas MAb R 1-20-5 reacts specifically with active renin *[23, 24]*. The MAbs were purified by $(NH_4)_2SO_4$ precipitation followed by chromatography with diethylaminoethyl cellulose *[25]*. Purity, as assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, was >98%. MAb R 3-36-16 was biotinylated *[26]*, and MAb R 1-20-5 was labeled with ^{125}I through use of a modified Chloramine-T method *[26, 27]*. The specific activity of the product was 740 GBeq/g of protein.

MAb F 55-3E8 and MAb F 55-4G1 were purchased as part of a commercially available IRMA kit for active renin (Renin III

Generation; ERIA Diagnostic Pasteur, Marnes La Coquette, France) *[28, 29]*. The MAbs were stored at 4 °C for no more than 6 weeks.

Avidin-coated plastic beads. Biotinylated bovine serum albumin was coupled to 8-mm-diameter polystyrene beads (Precision Plastic Ball Co., Chicago, IL), after which the beads were coated with avidin *[26]* and stored at 4 °C.

Renin standards. The International Reference Preparation of human kidney renin (IRP 68/356, 0.1 U of fully active renin per vial), was obtained from the National Institute for Biologic Standards and Control (Potters Bar, Herts., UK) *[30]*. We diluted it in 0.15 mol/L phosphate-buffered saline (PBS; 0.01 mol/L sodium phosphate, 0.15 mol/L NaCl), pH 7.4, containing 1 mL/L sheep serum and 1 g/L sodium azide. Highly purified recombinant human renin was a kind gift of K. Hofbauer (Ciba-Geigy, Basel, Switzerland). This standard, which we also diluted in the PBS diluent containing sheep serum and sodium azide, was calibrated against the IRP for human kidney renin with the enzyme kinetic PRC assay. In this assay renin is incubated at 37 °C with saturating concentrations of semipurified sheep angiotensinogen at pH 7.4, and the Ang I generated is measured by RIA *[13, 31]*. One nanogram of pure recombinant human renin corresponded with 1.4 ± 0.2 mU of the IRP (mean ± SD, 6 assays, 1–2 months apart). The renin standards were stored at −70 °C.

Renin inhibitor. The specific active-site-directed renin inhibitor Ro 42-5892 (Remikiren, mol. mass 727 Da) was obtained from Hoffmann-La Roche (Basel, Switzerland) *[22]*. The K_i value for the reaction with human renin is 3×10^{-10} mol/L. The inhibitor was dissolved in 0.15 mol/L PBS, pH 7.4, to give a concentration of 10^{-3} mol/L. Only freshly prepared solutions of Remikiren were used.

Angiotensinogen. Angiotensinogen was prepared from plasma of nephrectomized sheep *[32]*. The semipurified preparation was dialyzed against 0.15 mol/L PBS, pH 7.4, and neomycin sulfate was added (final concentration, 2 g/L). The final preparation had an angiotensinogen concentration of 1.2×10^{-6} mol/L, as determined from the maximal quantity of Ang I that was generated during incubation at pH 7.4 and 37 °C with a high concentration (10 000 mU/L) of the IRP for human kidney renin. The angiotensinogen stock solution was stored at −70 °C.

Sepharose-bound trypsin. Trypsin from bovine pancreas (T-8003; Sigma, St. Louis, MO) was used for the proteolytic activation of prorenin. After coupling the trypsin to CBr-activated Sepharose (Sepharose 4B; Pharmacia, Uppsala, Sweden), 25 mg of trypsin to 1 g of Sepharose *[13]*, we suspended 1 g of this preparation in 10 mL of 0.01 mol/L sodium phosphate buffer, pH 7.0. The Sepharose-bound trypsin could be stored at 4 °C for at least 2 years without loss of activity.

METHODS

Proteolytic activation of prorenin. Prorenin was converted to renin by the addition of trypsin. We mixed 200 μL of Sepharose-bound trypsin suspension (0.5 mg of trypsin) with 1 mL of plasma, and incubated the mixture for 24 h at 4 °C *[13]*. The trypsin was then removed by centrifugation.

Nonproteolytic activation of prorenin. Prorenin was treated with the active-site-directed renin inhibitor, Remikiren, to change the molecular conformation of prorenin from the closed (inactive) form into the open (active) form *[21]*. This process removed the prosegment of prorenin from the cleft between the two lobes of the molecule harboring renin's active site. MAbs that are specific for active renin and do not recognize the closed form of prorenin will also react with the open form of prorenin *[21]*. We therefore refer to the Remikiren-induced conformational change of prorenin as "activation," despite the fact that the prorenin–Remikiren complex is devoid of enzymatic activity. To activate all the prorenin in plasma *[21]*, we added 20 μL of Remikiren (10^{-3} mol/L) to 200 μL of plasma and incubated the mixture for 24 h at 4 °C.

Enzyme kinetic assays of naturally occurring renin and trypsin-activated prorenin. To measure PRC, we used our in-house assay, as described elsewhere *[8, 13]*. In short, we added 250 μL of sheep angiotensinogen solution to 50- to 250-μL aliquots of plasma, and adjusted the volume to 500 μL with 0.15 mol/L PBS, pH 7.4. After addition of protease inhibitors—10 μL of 0.34 mol/L 8-hydroxyquinoline sulfate, 5 μL of 0.287 mol/L phenylmethylsulfonyl fluoride in ethanol, 10 μL of 0.5 mol/L disodium EDTA, and 10 μL of aprotinin (100 kallikrein-inhibiting units)—we incubated the mixture at 37 °C for 1 h. The final concentration of sheep angiotensinogen in the incubation mixture was 6×10^{-7} mol/L (K_M 2×10^{-7} mol/L). Only incubations in which <5% of angiotensinogen was hydrolyzed were accepted for calculating the concentration of naturally occurring renin or trypsin-activated prorenin. Under these circumstances Ang I generation is linear for at least 1 h. Parallel incubations at 0 °C served as blanks. The generated Ang I was measured by RIA *[31]*. The lower limit of detection of the Ang I RIA was 5 fmol of Ang I in an assay tube containing 50 μL of incubate. Results of the measurements of naturally occurring renin and trypsin-activated prorenin are expressed as mU/L, as calibrated with the IRP of human kidney renin. Under the conditions of the assay, 1 mU of renin generates 163 pmol (212 ng) of Ang I per hour.

IRMAs of naturally occurring renin and Remikiren-activated prorenin. MAb 3-36-16 and MAb 1-20-5 were used in the newly developed IRMAs we validated in the present study, the former chosen because of its high affinity for both prorenin and renin *[23]*, the latter because of its specificity for renin (see Table 1) *[24]*. Binding of the antigen to MAb 3-36-16 did not interfere with the binding to MAb R 1-20-5 *[23]*.

We pipetted into polystyrene tubes 200 μL of plasma or of a series of dilutions of pure recombinant human renin and mixed

this with 100 μL of an equivolume mixture of biotinylated MAb R 3-36-16 (0.5 mg/L) and radiolabeled MAb R 1-20-5 (~250 000 counts/min). The plasma samples were either untreated or had been treated with Remikiren to activate prorenin before the assay. An avidin-coated bead was added to each tube, and the mixtures were incubated for 24 h at room temperature without shaking. Subsequently the beads were washed three times with 2 mL of 0.15 mol/L PBS, pH 7.4, containing 1 g/L sodium azide, and the radioactivity bound to them was counted in a gamma counter. The concentration of prorenin was calculated by subtracting the result obtained before Remikiren activation from that after activation. Results of the measurements of renin and Remikiren-activated prorenin are expressed as mU/L, also calibrated with the IRP of human kidney renin.

We also measured renin with the commercially available IRMA kit for active renin from ERIA Diagnostic Pasteur *[29]*. This kit uses MAb F 55-3E8 and MAb F 55-4G1 (see Table 1), and the results are expressed as ng/L.

Assay of angiotensinogen. The concentration of angiotensinogen in plasma was determined as the maximal quantity of Ang I that was generated during incubation at pH 7.4 and 37 °C of plasma having a high content of human kidney renin (10 000 mU/L) *[8]*. Results are expressed as mol/L.

CONTROL SUBJECTS AND PATIENTS

The control subjects were 100 healthy normotensive Caucasians (50 women, 50 men), all university personnel or students, ages 19–62 years (median 39 years), who were on a normal-sodium diet. Results for these normal subjects were compared with those from the following groups of individuals: subjects with essential hypertension (n = 29, 14 women), ages 27–75 years (median 52 years); patients with hypertension associated with unilateral renal artery stenosis (n = 28, 13 women), ages 24–72 years (median 62 years); patients with primary aldosteronism (Conn syndrome, n = 4, 3 women), ages 46–51 years; healthy women in the third trimester of pregnancy (n = 11), ages 19–39 years (median 30 years); pregnant women with hypertension due to preeclampsia (n = 13), ages 24–40 years (median 32 years); women taking an estrogen-containing oral contraceptive for at least 6 months (n = 13), ages 21–39 years (median 25 years); patients with insulin-dependent diabetes mellitus (n = 17, 8 women), ages 34–66 years (median 51 years); and women participating in an in vitro fertilization program (n = 19), ages 29–40 years (median 34 years).

All subjects with essential hypertension or hypertension associated with renal artery stenosis had undergone renal angiography and renin sampling from the renal vein. Renal artery stenosis was defined as narrowing of the lumen by 60% or more on the arteriogram. In patients with Conn syndrome the presence of an adrenal adenoma was confirmed at surgery. The gestational age of the healthy pregnant women was 25–37 weeks (mean 32 weeks). The gestational age of the women with preeclampsia was 30–38 weeks (mean 35 weeks). The patients with preeclampsia had a diastolic blood pressure of 100 mmHg or higher, combined with proteinuria of 500 mg/L or more. None of these women had preexisting hypertension or renal or heart disease, and their serum creatinine concentrations were normal. None of these patients had been treated with antihypertensives, and all were normotensive 6 weeks after delivery. All patients with diabetes had "micro"-albuminuria (urinary albumin excretion between 30 and 300 mg/24 h) and background or proliferative retinopathy. The duration of their diabetes was 13 (4–45) years (median and range). The diabetic patients were taking insulin but not any other medication.

The women participating in a program for in vitro fertilization were treated, from day 13 before ovum pick-up, with a short-acting analog of gonadotropin-releasing hormone, leuprolide (Lucrin; Abbott, Amstelveen, The Netherlands), 1 mg a day subcutaneously, until ovulation induction. From day 12 before ovum pick-up, human follicle-stimulating hormone (hFSH) and human lutein-stimulating hormone (hLH) were administered, two ampoules of Humegon 75 (75 IU of hFSH and 75 IU of hLH; Organon, Oss, The Netherlands) being injected intramuscularly twice a day. Stimulation was monitored by serum estrogen measurements and transvaginal ultrasound. Ovulation was induced by intramuscular injection of 10 000 IU of human chorionic gonadotropin (Pregnyl; Organon), 6 days after serum estrogen concentrations reached 270 ng/L (~10^{-9} mol/L). Ovum pick-up and follicular fluid collection were performed 34–35 h later.

COLLECTION OF SAMPLES

An indwelling catheter was inserted into a forearm vein. Subjects then rested quietly in the recumbent position for 45 min before blood samples were drawn. Blood was collected into tubes containing trisodium citrate, 0.2 mL of 0.646 mol/L citrate per 10 mL of blood. In the control subjects blood was collected not only into citrate but also into tubes containing disodium EDTA (0.1 mL of 0.5 mol/L EDTA per 10 mL of blood) and into tubes without anticoagulant. Within 30 min after sampling, the blood was centrifuged at 3000*g* for 10 min at room temperature, and plasma and serum were stored in 1-mL aliquots at −20 °C. Shortly before the assay, the samples were rapidly thawed and kept at room temperature, to avoid cryoactivation of prorenin.

Ovum pick-up and follicular fluid collection were performed with an ultrasound-guided single-lumen follicle aspiration needle (17-gauge, 35 cm long; William A. Cook Europe, Bjaeverskov, Denmark). After separation of the oocyte, follicular fluid was immediately centrifuged for 10 min at room temperature and 2000*g*. Follicular fluid samples were stored in polystyrene tubes at −20 °C.

STATISTICS

Plasma renin and prorenin results have a log normal distribution. Analyses of variance, *t*-tests, and regression analyses were performed after logarithmic transformation of data. The "normal" reference values were calculated according to the recommendations of Solberg *[33]*.

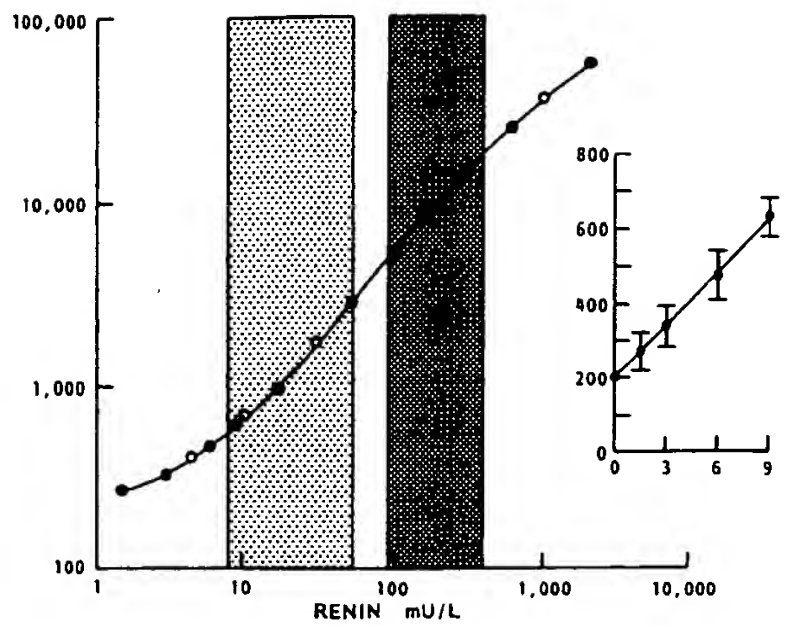

Fig. 1. Calibration line of the IRMA of renin: counts per minute (*ordinate*) plotted against the concentration of renin, mU/L.

O, IRP of human kidney renin; ●, recombinant human renin standard. *Light gray area (left)*: normal reference limits for plasma concentrations of renin; *dark gray area (right)*: normal reference limits for plasma concentrations of prorenin. *Inset*: calibration line for low concentrations of renin (mean ± 3 SD).

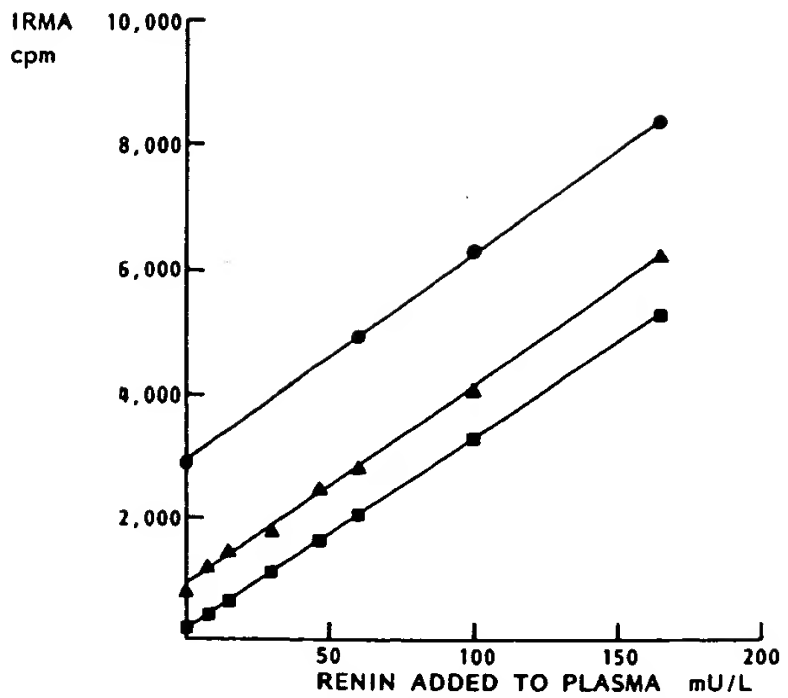

Fig. 2. Effects of adding increasing amounts of recombinant human renin to two plasma pools (endogenous renin 21 mU/L ▲ and 88 mU/L ●) or to buffer (■, 0.15 mol/L PBS, pH 7.4, containing 1 mL/L sheep serum).

Ordinate: IRMA results for renin.

Results

PERFORMANCE CHARACTERISTICS

Calibration line. In the absence of renin, but in the presence of the biotinylated renin antibody MAb R 3-36-16, <0.1% of the radiolabeled renin antibody MAb R 1-20-5 was bound to the avidin-coated beads. The first-order rate constant for the binding of radiolabeled MAb R 1-20-5, in the presence of renin, was 0.2 h^{-1} at room temperature. Thus, the incubation time of 24 h we used was sufficient to obtain maximal binding. Binding was not increased by using a two-step procedure in which biotinylated MAb R 3-36-16 and the avidin-coated bead were incubated for 24 h as a first step, and the bead was then washed and incubated with active renin and radiolabeled MAb R 1-20-5 for 24 h as a second step.

Figure 1 depicts the calibration lines of the IRMA for both the human kidney renin IRP and the recombinant human renin standard. The IRMA results, expressed as counts per minute, were plotted against the concentration of renin, expressed as mU/L. Results were identical for the two renin standards. The quantity of radiolabeled MAb R 1-20-5 trapped by renin was proportional to renin concentration over a wide range (1.5–3000 mU/L). A renin concentration of 30 000 mU/L still showed no high-dose hook effect (data not shown).

Parallelism was tested by adding a series of dilutions of recombinant human renin to plasma (1 volume of recombinant renin to 10 volumes of plasma). When the IRMA results, expressed as counts per minute, were plotted against the concentration of recombinant renin, expressed as mU/L, the results paralleled the calibration line for recombinant renin in buffer (Fig. 2).

Linearity was tested by preparing 2- to 128-fold dilutions of various plasma samples with renin concentrations ranging from 9.8 to 1630 mU/L. Plots of the IRMA results against the dilution factor gave straight lines parallel to the expected line (Fig. 3).

In the IRMA of renin we developed, we used recombinant human renin as the standard, which was calibrated against the IRP for human kidney renin. The IRMA kit for renin from ERIA Diagnostics Pasteur, which is now commercially available,

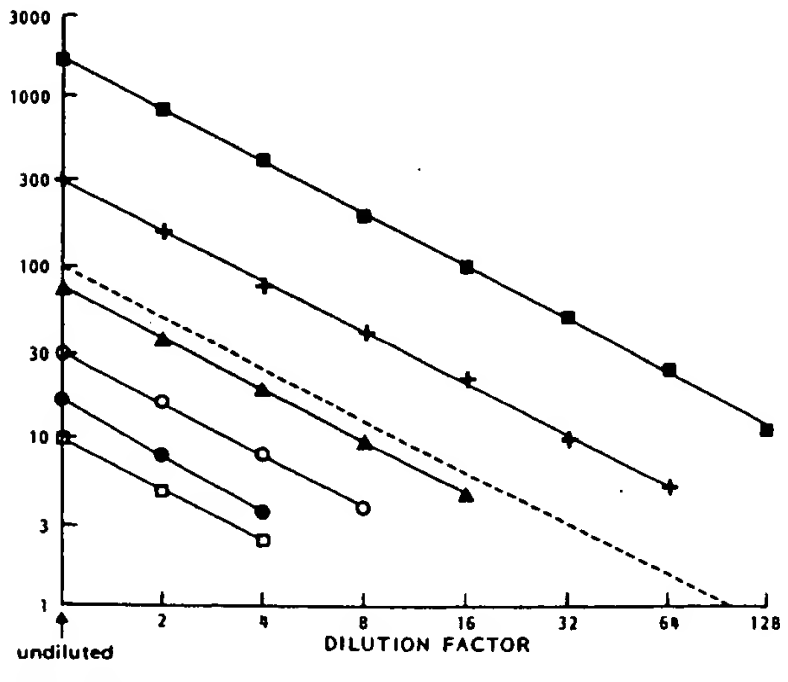

Fig. 3. Plasma dilution tests for plasmas from a patient with essential hypertension (□), a normal male control subject (●), a patient with diabetes mellitus (○), a patient with essential hypertension treated with enalapril (▲), an untreated patient with renovascular hypertension (+), and a patient with renovascular hypertension treated with enalapril (■).

Ordinate: IRMA results for renin, mU/L. The *dotted line* is the theoretical line for a plasma with a concentration of renin of 100 mU/L.

Table 2. Recovery of human kidney renin standard added to plasma.

Plasma pool	Plasma renin conc, mU/L: Added to plasma	Endogenous	Total measured	Recovery, %
Patients with essential hypertension	2.5	8.2 (0.6)[a]	10.5 (1.1)[a]	98 (13)[a]
	5	8.2 (0.6)	13.6 (1.0)	103 (9)
	10	8.2 (0.6)	17.9 (1.2)	97 (9)
Women taking estrogen-containing oral contraceptive	10	11.1 (0.9)	20.7 (1.4)	98 (8)
Control subjects	50	25.5 (1.6)	77.7 (4.6)	103 (9)
Patients with renovascular hypertension	100	240 (11.6)	338 (18.4)	98 (9)

[a] Results are means and SD. n = 10 for each pool.

uses as a working calibrator serum from baboons treated with furosemide *[38]*. Results obtained with our IRMA in 51 plasmas with renin concentrations ranging from 4.5 to 1660 mU/L showed a linear correlation with the Pasteur IRMA kit (r^2 = 0.99). The regression line equation was $y = 0.9909x + 0.4032$, where y = log renin by our IRMA (mU/L) and x = log renin by the Pasteur IRMA kit (ng/L); 1 mU in our assay corresponded to 0.4 ng in the Pasteur assay.

Analytical recovery. The IRP for human kidney renin was added, in final concentrations of 2.5, 5, and 10 mU/L, to a plasma pool already containing renin at 8.2 mU/L. The renin IRP was also added in a final concentration of 50 mU/L to a plasma pool with a concentration of renin of 25.5 mU/L, and in a final concentration of 100 mU/L to a plasma pool with a concentration of renin of 240 mU/L. The IRMA results (Table 2) demonstrate excellent analytical recovery of both low and high quantities of added renin.

MAbs R 3-36-16 and R 1-20-5 are capable of inhibiting the Ang I-generating activity of renin (Table 1). Angiotensinogen might therefore interfere with the binding of renin to these antibodies. To find out whether such interference really exists, we added human kidney renin IRP in a final concentration of 10 mU/L to a plasma pool having a high concentration of angiotensinogen. This pool, obtained from women taking an estrogen-containing oral contraceptive, had an angiotensinogen concentration of 6.2×10^{-6} mol/L, about six times normal, and a concentration of renin of 11.1 mU/L. Estrogen is known to stimulate angiotensinogen production from the liver; however, angiotensinogen, even at this high concentration, did not influence the IRMA results (see Table 2).

Assay variations. Reproducibility was estimated by determining intra- and interassay CVs in low-, medium-, and high-concentration renin plasmas. Interassay CV was determined from 10 consecutive assays, 3–4 days apart. Results are summarized in Table 3. The interassay CV of the IRMA of renin was 18% in a plasma with a low renin content (4.0 mU/L), 13% in a medium-range plasma (22.2 mU/L), and 7% in a high-range plasma (209 mU/L). At medium and high concentrations of renin, the interassay CV of the IRMA was similar to that of the enzyme kinetic PRC assay. At low concentrations of renin, the interassay CV for the IRMA was somewhat greater than for the enzyme kinetic assay. After samples were activated with Remikiren, the interassay CV for prorenin calculated from the IRMA was ~8%, somewhat lower than with the enzyme kinetic PRC assay after activation with trypsin.

Assay sensitivity. The detection limit of the renin IRMA, defined as 3 SD above the mean for the zero standard (n = 20), was 2.1 mU/L. This value was obtained with a tracer on the day of its expiration (8 weeks after labeling). With fresh tracer, the detection limit was 1.5 mU/L. The functional sensitivity, i.e., the minimum concentration that can be measured from assay to assay with <20% CV *[34]*, was 4.0 mU/L or better (see Table 3).

Enhancement of prorenin immunoreactivity with Remikiren. Fig. 4 shows the IRMA result for a normal plasma pool that was treated for various time intervals (up to 72 h) with the renin inhibitor, Remikiren, at a final concentration of 10^{-4} mol/L, before incubation with the antibodies and the avidin-coated bead, as described in *Materials and Methods*. After treatment with Remikiren, the maximal immunoreactivity plateaued within 24 h, and this plateau was at the same concentration value as the maximal value reached with Sepharose-bound trypsin. The immunoreactivity was 60% of the maximum when plasma was incubated with the antibodies and the avidin-coated bead immediately after the addition of Remikiren. This high immunoreactivity, despite the short pretreatment with Remikiren, results from the activation of prorenin by the inhibitor during incubation with the antibodies for 24 h.

By adding Remikiren in a final concentration of 10^{-4} mol/L to a series of dilutions of renin standards similar to those shown in Fig. 1, and by comparing the IRMA results of these Remikiren/renin mixtures with the IRMA results of the same

Table 3. Intraassay and interassay CVs determined in plasma samples.

Plasma conc, mU/L	Plasma code	Intraassay CV, % (n = 6): Enzyme kinetic	Intraassay CV, % (n = 6): IRMA	Interassay CV, % (n = 10): Enzyme kinetic	Interassay CV, % (n = 10): IRMA
Renin					
4.0	A	7.3	7.5	12.1	18.3
8.7	B	6.9	5.9	10.2	12.0
22.2	C	8.2	8.2	12.3	13.3
67.4	D	5.3	5.3	6.7	8.3
209	E	3.8	3.8	7.9	7.0
Prorenin					
60.3	A	10.2	7.0	14.8	7.1
320	D	4.2	4.2	12.5	7.8
640	E	8.0	8.0	11.6	6.9

Data are means.

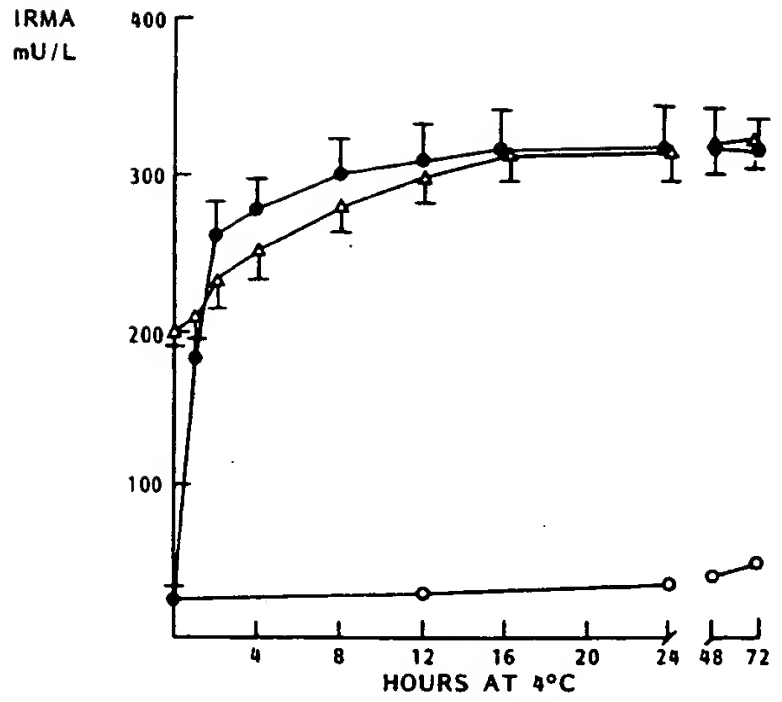

Fig. 4. Activation of prorenin in plasma, as determined with results obtained with IRMA: (○) cryoactivation at 4 °C; (△) incubation with 10^{-4} mol/L Remikiren at 4 °C; (●) incubation with 0.5 g/L Sepharose-bound trypsin at 4 °C.

Results are means and SD, n = 5. Note that the measured renin in the presence of Remikiren was already high at time zero, because of the activation of prorenin by Remikiren during the 24 h of incubation with the MAbs.

renin dilutions without Remikiren, we determined that the binding of the antibodies to renin was not influenced by Remikiren (data not shown).

Sample stability and effect of anticoagulant. Citrate- and EDTA-treated plasma as well as serum were obtained from blood collected from 10 normal subjects. The IRMA results for renin and calculated prorenin in citrate- and EDTA-treated plasma and in serum were not significantly different. In citrate- and EDTA-treated plasma and in serum, concentrations of renin and prorenin were stable at room temperature for 24 h. In whole blood, renin and prorenin were also stable at room temperature for 24 h. We routinely centrifuged the blood samples within 30 min after collection. Centrifugation of normal citrate- or EDTA-treated plasma (n = 10 for each anticoagulant) at 3000*g* for 30 min at 4 °C, instead of room temperature, did not cause detectable cryoactivation of prorenin. Freezing and thawing of the samples twice led to a small but significant increase in the measured concentration of renin, from 13.3 to 15.4 mU/L (mean values in 10 plasmas from normal subjects, $P < 0.05$).

CLINICAL VALIDATION OF RENIN AND PRORENIN IRMAS

Comparison between the IRMA of renin and the enzyme kinetic PRC assay. The IRMA results were often somewhat higher than the results of the enzyme kinetic assay. The largest differences were observed in pregnant women and in patients with diabetes (Table 4). In these subgroups prorenin was markedly increased, whereas renin was normal or only slightly increased. The higher IRMA results may be caused by activation of prorenin during the assay or by some cross-reactivity of MAb R 1-20-5 with prorenin. This would mean that the concentration of renin measured by IRMA is in fact an overestimation of the true value. In Table 5 the difference between the concentrations of renin measured by IRMA and by the enzyme kinetic assay is expressed both as a percentage of the renin measured by IRMA and as a percentage of the prorenin value. The latter may be taken as a measure of the fraction of prorenin that is detected in the IRMA as renin. In the various patients' groups, this fraction was 0.3–1.8%. Thus our IRMA appears to overestimate plasma renin by ≤12% on average in most subject groups. In pregnant women and in diabetics, renin is overestimated in the IRMA by ~30%. These are also the two groups with the highest prorenin concentrations in plasma.

Results of the two assays were linearly correlated (Fig. 5), not only when all subject groups were taken together but also when the different subgroups, including those with low renin content, were separately analyzed; r^2 values in the individual subgroups ranged from 0.58 to 0.99. The slopes of the regression lines of the individual subgroups were similar to the slope of the line of identity.

Measurements of renin in ovarian follicular fluid from gonadotropin-treated women showed results that were about three times higher with IRMA than with the enzyme kinetic assay (Table 4). The difference between the amounts of renin measured by the two assays was 1.9% of the prorenin concentration, which is similar to what was found in plasma.

To examine whether the IRMA is sensitive enough to detect the effect of body position on the plasma concentration of renin, we measured specimens from a subgroup of 56 normal subjects (25 women, 29 men) ranging in age from 19 to 46 years (median 32 years), while they were supine for 45 min and after they had been upright for 1 h. Renin in this group was 17.9 mU/L (geometric mean) in the supine position, but was 55% ± 26% (mean ± SD) greater in the upright position ($P < 0.0001$).

In many clinical centers the renal vein/renal artery ratios of renin across the right and left kidney are measured to assess the functional importance of a renal artery stenosis demonstrated on the arteriogram. Fig. 6 compares the ratios measured with the IRMA and those measured with the enzyme kinetic PRC assay. Agreement between the two assays was excellent.

Comparison between the IRMA after activation of prorenin with Remikiren and the enzyme kinetic PRC assay after activation of prorenin with trypsin. Results for calculated prorenin in the two assays were closely correlated, both at low and high concentrations of prorenin (Fig. 7). The small variations around the regression line can, to a large extent, be explained by the interassay CVs of the two assays. In some groups of subjects, the results by the Remikiren-enhanced IRMA were somewhat higher than by the enzyme kinetic assay after activation with trypsin—i.e., in plasma from control subjects, in plasma from pregnant women with or without preeclampsia, and in plasma and ovarian follicular fluid from gonadotropin-stimulated women.

The concentrations of plasma prorenin on the day of ovum pick-up, whether calculated from the results obtained with

Table 4. Plasma concentrations of renin and prorenin in control subjects and in various groups of patients.

Patient groups	n	Renin, mU/L[a]		Calculated prorenin, mU/L[a]		Calculated prorenin % of renin + prorenin sum[b]	
		Enzyme kinetic	IRMA	Enzyme kinetic	IRMA	Enzyme kinetic	IRMA
Control subjects	36	22.1	25.6[c]	208	223[c]	89.4 (2.9)	89.2 (3.1)
		10.1–45.7	11.3–44.6	139–352	123–405		
Men	22	23.0	26.8[c]	209	232[c]	89.5 (3.7)	89.2 (3.5)
		10.1–43.2	16.7–44.6	150–332	156–405		
Women	14	20.8	23.6	196	209	89.4 (4.8)	89.2 (3.9)
		10.3–45.7	11.3–46.1	139–313	123–337		
Women patients							
3rd trimester of pregnancy	13	32.3	45.6[c]	811	882[c]	95.9 (1.7)	94.9 (1.9)
		20.5–63.9	29.9–69.7	441–1590	467–1685		
Preeclampsia	13	18.0	27.1[c]	598	662[c]	96.1 (2.9)	94.2 (2.9)
		5.3–61.4	8.2–70.9	292–990	299–1168		
Taking an estrogen-containing contraceptive	13	13.0	14.0	115	110	89.4 (3.2)	87.9 (2.4)
		3.4–41.4	3.8–35.3	42.6–263	42.4–228		
Gonadotropin-treated							
Plasma	19	27.5	32.0[c]	1070	1326[c]	97.4 (0.9)	97.5 (0.8)
		11.6–63.5	9.0–79.0	341–2618	326–3980		
Follicular fluid	19	237	607[d]	15 653	20 058[c]	98.2 (1.3)	96.8 (1.4)
		103–774	307–1076	8041–52 959	10 434–54 444		
Insulin-dependent diabetes mellitus	17	20.1	30.4[c]	606	638	96.4 (2.0)	95.1 (1.9)
		7.8–76.6	10.4–58.5	222–2585	188–2724		
Essential hypertension							
No therapy	15	12.9	14.8	141	142	91.1 (2.8)	90.2 (2.8)
		5.9–33.3	7.0–34.9	56.1–350	63.0–334		
Enalapril-treated	14	62.3	64.3	270	283	78.3 (8.3)	79.5 (8.1)
		16.7–243	16.4–262	110–654	134–698		
Renovascular hypertension							
No therapy	14	86.1	91.5	443	472	82.9 (5.8)	82.8 (5.1)
		15.3–477	15.1–389	150–1225	158–1495		
Enalapril-treated	14	469	482	590	646	55.3 (15.3)	56.4 (15.5)
		171–1888	184–1798	161–1659	167–1607		
Conn syndrome	4	5.5	8.3	57.4	59.1	91.8 (1.8)	88.9 (4.2)
		3.4–5.8	4.1–9.4	44.0–64.1	37.2–896		

[a] Geometric means and ranges.
[b] Mean (SD).
[c,d] Significantly different from enzyme kinetic assay results; [c] $P < 0.05$, [d] $P < 0.01$.

IRMA after Remikiren or with the enzyme kinetic assay after trypsin, correlated with the number of follicles ($r^2 = 0.32$ and $r^2 = 0.31$, respectively; $P < 0.05$ by Spearman rank test), with the plasma estradiol concentrations ($r^2 = 0.52$ and $r^2 = 0.50$; $P < 0.01$), and with the total amount of ovarian follicular fluid ($r^2 = 0.33$ and $r^2 = 0.30$; $P < 0.05$). The concentrations of renin in plasma, measured without pretreatment by both the IRMA and the enzyme kinetic assay, did not correlate with the aforementioned variables. These results for renin and prorenin in ovarian follicular fluid are in agreement with published data obtained with enzyme kinetic assays *[9]*.

Reference values. The reference values for renin and calculated prorenin that were determined with our IRMA in plasma from normal control subjects are given in Table 6. The amount of prorenin in normal plasma is ~9 times the amount of renin. Both renin and prorenin appeared to be present in higher concentrations in men than in women.

Discussion

The renin IRMA is a one-step assay, in contrast to the two-step enzyme kinetic assays. The latter require an incubation step for generation of Ang I, which is then measured by RIA. Another advantage of the IRMA over the enzyme kinetic assays is the wide range over which the IRMA results, expressed as counts per minute, are linearly correlated with the concentrations of active renin. The linearity of our IRMA extends to a renin concentration of 3000 mU/L. The Pasteur kit has a working range to 320 ng/L (corresponding to 800 mU/L; see *Results*). When the enzyme kinetic assay is used for samples with a high concentration of renin, one must often repeat the assay after the samples have been properly diluted.

In our hands the interassay CV of IRMA was ~10% for plasma samples with medium or high concentrations of renin, and ~20% for low renin concentrations. Until now, most laboratories have measured renin in plasma with the enzyme kinetic PRA assay; however, between-laboratory variations in

Table 5. Overestimation of renin measurements by the IRMA.

		Mean (SD) overestimation	
Patient group	n	% of IRMA result	% of prorenin[a]
Control subjects	36	10.3 (24.1)	1.1 (2.7)
Men	22	11.1 (23.1)	1.4 (2.6)
Women	14	8.9 (25.4)	0.6 (2.9)
Women patients			
3rd trimester of pregnancy	13	28.1 (12.7)	1.5 (0.9)
Preeclampsia	13	34.7 (15.7)	1.5 (0.9)
Taking an estrogen-containing contraceptive	13	9.1 (19.4)	0.5 (2.8)
Gonadotropin-treated			
Plasma	19	10.4 (25.1)	0.3 (0.7)
Follicular fluid	19	57.4 (15.3)	1.9 (0.9)
Insulin-dependent diabetes mellitus	17	31.7 (17.9)	1.6 (1.2)
Essential hypertension			
No therapy	15	12.0 (12.8)	1.2 (1.3)
Enalapril-treated	14	2.3 (13.5)	0.9 (2.1)
Renovascular hypertension			
No therapy	14	6.9 (11.2)	1.1 (2.6)
Enalapril-treated	14	3.4 (10.8)	1.9 (2.3)
Conn syndrome	4	29.5 (9.5)	3.9 (2.1)

[a] Presented to examine the possibility that the difference between the IRMA results for renin (in the absence of Remikren) and the results of the enzyme kinetic assay is caused by comeasurement of a small fraction of prorenin in the IRMA of renin. Prorenin is therefore defined here as the difference between the results obtained with the IRMA in the presence of Remikiren and the results of the enzyme kinetic assay.

PRA results have reportedly been as high as 65% *[35]*. The simplicity of the IRMA method is likely to reduce these interlaboratory variations. Another important advantage of the IRMA over the PRA assay is also the direct comparison with a renin reference material, which is routinely used in each assay batch, thereby permitting ready comparison of results from different laboratories.

In some situations it is useful to perform parallel measurements with IRMA and the enzyme kinetic assay. For example, treatment of hypertensive subjects with a renin inhibitor causes a decrease in plasma renin measured as enzymatic activity, but an increase in renin measured as immunoreactivity *[36]*. From such parallel renin measurements, the extent of in vivo renin inhibition can be estimated.

The IRMA also has its limitations. Based on our data on the reproducibility of the IRMA of renin in plasmas with low and low-normal concentrations, the lower limit of the working range (functional sensitivity) is 4.0 mU/L. The working range of the enzyme kinetic PRC assay, however, can be extended to even lower concentrations by prolonging the Ang I generation time *[5]*, and very low amounts of renin activity can be detected by similar manipulations with the PRA assay. It has not been established, however, whether the rate of Ang I generation is constant during such long incubation times. Despite its lower sensitivity, the IRMA is capable of identifying subjects with abnormally low concentrations of plasma renin, e.g., patients with primary hyperaldosteronism and some patients with essential hypertension. Even at such low concentrations of renin, the IRMA results correlated with the results of the enzyme kinetic PRC assay. The lower limit of the normal reference range for the IRMA was 7.7 mU/L, well above the functional sensitivity of the assay. Apparently, therefore, the IRMA we describe is sufficiently sensitive for clinical use.

That the results of the renin IRMA were somewhat higher than the results of the enzyme kinetic PRC assay should also be discussed. Presumably, the enzyme kinetic assay is more accurate, in that it measures only active renin and not prorenin or any renin-like substance incapable of reacting with angiotensinogen to form Ang I. The higher IRMA results may then be due to inadvertent nonproteolytic activation of prorenin during the 24 h of incubation with the renin antibodies. Nonproteolytic prorenin activation occurs not only at low temperatures (cryoactivation) and low pH (acid-activation) but also at room temperature and neutral pH *[19]*. The higher IRMA results may also reflect some cross-reactivity of MAb R 1-20-5 with prorenin. In an alternative theory, perhaps the IRMA detects an active (open) form of prorenin that is not measured by the enzyme kinetic assay. The in vitro acid- or cryoactivated (open) forms of prorenin are known to return to their inactive (closed) conformation during incubation at neutral pH and 37 °C *[18, 19]*. Whether such open forms of prorenin are present in vivo in circulating plasma is, however, unknown.

Our results in healthy control subjects and various groups of patients indicate that 0.3–1.8% of prorenin was measured by the IRMA as renin. Plasma prorenin is high in pregnancy and in diabetes mellitus. In such conditions, the incorrect measurement of even a small percentage of prorenin as renin will lead to a relatively large percentage increase in the measured concentration of renin, because the real concentration of renin is much lower than that of prorenin. When prorenin is present at 9 times the concentrations of renin, as is the case in normal plasma, measuring 2% of the prorenin as renin in the IRMA will yield results that are ~15% too high. When the prorenin concentration is 25 times that of renin, as in pregnancy and diabetes, the renin concentration measured with IRMA will be 30% too high. Because the prorenin concentration in ovarian follicular fluid from gonadotropin-treated women is greatly higher than the concentration of renin, we can easily see why the concentrations of renin measured with IRMA in this fluid were 2.5 times higher than those measured with the enzyme kinetic assay, even at the low (2%) cross-reactivity in the IRMA.

Despite the differences between the two assays, their results in plasma were significantly correlated, not only when all subject groups were taken together but also within the different groups, in both the low- and high-renin ranges. Moreover, the regression lines for these subgroups were parallel to the line of identity. Also, the interassay CV of the IRMA of renin was not higher than the interassay CV of the enzyme kinetic assay at medium and high concentrations of plasma renin. The interassay CV being somewhat higher by the IRMA than by the

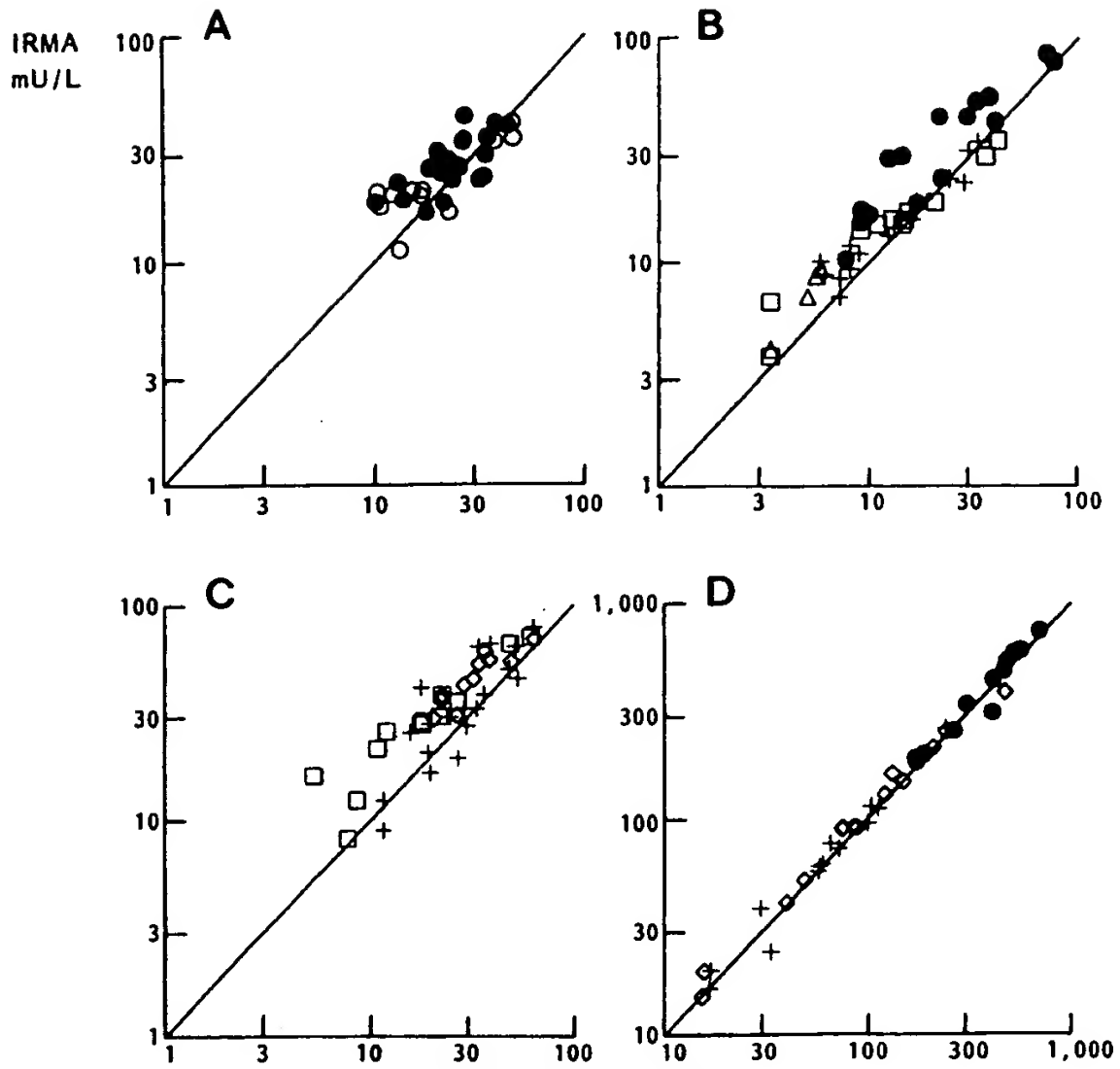

Fig. 5. Comparison between the plasma concentrations of renin measured with IRMA and the enzyme kinetic PRC assay in (*A*) normal subjects (men ●, women ○); (*B*) patients with Conn syndrome (△), diabetes mellitus (●), or untreated essential hypertension (+), and women taking an estrogen-containing oral contraceptive (□); (*C*) pregnant women (◇) or women with preeclampsia (□) or receiving gonadotropin stimulation (+), (*D*) patients with renovascular hypertension—untreated (+) or treated with enalapril (●)—or with essential hypertension treated with enalapril (◇).

Line of identity is shown in all panels. Least-squares regression equations were obtained after logarithmic transformation of the results. The *y*-intercept is given as the antilog. Normal subjects: n = 36, slope = 0.89, intercept = 2.5, r^2 = 0.58 (P <0.0001); Conn syndrome patients: n = 4, slope = 1.28, intercept = 0.6, r^2 = 0.98 (P <0.01); women taking estrogen-containing oral contraceptive: n = 13, slope = 0.76, intercept = 1.2, r^2 =0.96 (P <0.0001); essential hypertension, untreated: n = 15, slope = 0.99, intercept = 1.7, r^2 = 0.94 (P <0.0001); diabetes mellitus: n = 17, slope = 1.01, intercept = 2.7, r^2 = 0.88 (P <0.0001); pregnant women: n = 13, slope = 0.92, intercept = 2.1, r^2 = 0.80 (P <0.001); preeclampsia: n = 13, slope = 1.08, intercept = 2.9, r^2 = 0.84 (P <0.0005); gonadotropin-stimulated women: n = 19, slope = 0.99, intercept = 1.2, r^2 = 0.84 (P <0.0001); renovascular hypertension, untreated: n = 14, slope = 0.95, intercept = 1.1, r^2 = 0 .99 (P <0.0001); renovascular hypertension treated with enalapril: n = 14, slope = 0.99, intercept = 1.0, r^2 =0.98 (P <0.0001); essential hypertension treated with enalapril: n = 14, slope = 1.05, intercept = 1.0, r^2 = 0.97 (P <0.0001).

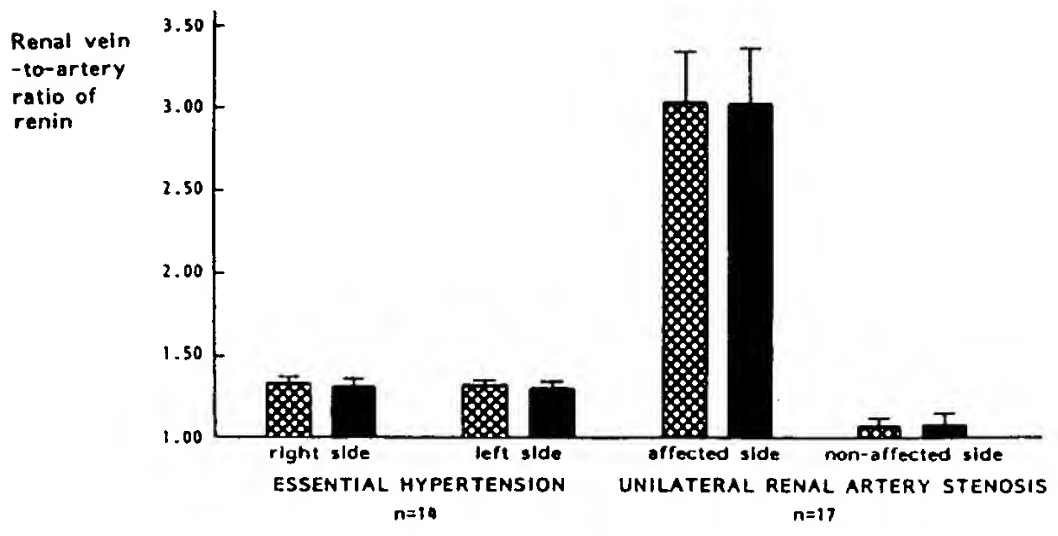

Fig. 6. The renal vein-to-artery ratios of renin in patients with essential hypertension and patients with unilateral renal artery stenosis.

Hatched bars: results obtained with the enzyme kinetic PRC assay; *black bars*: results obtained with the renin IRMA. Results are means and SE. Results of the two assays were not significantly different. Ratios on the affected side in patients with renal artery stenosis were higher than the ratios on both sides in essential hypertension (P <0.001). Ratios on the nonaffected side in patients with renal artery stenosis were lower than the ratios in essential hypertension (P <0.001).

enzyme kinetic assay in plasmas with low renin concentrations is probably due to the fact that these low concentrations are close to the detection limit of the IRMA. In addition, the interindividual variability of renin concentrations in the subject groups we studied was similar for IRMA and the enzyme kinetic assay, despite the fact that the samples from these groups had widely different concentrations of prorenin. Apparently, comeasurement of some prorenin by the IRMA did not lead to an unacceptably high variability of the results. We therefore conclude that the overestimation of renin by IRMA is not important in clinical practice.

The IRMA we have described utilizes a unique method for activating prorenin—with use of the active-site-directed renin inhibitor, Remikiren. Both the renin and prorenin concentrations can be calculated from the same calibration line. To measure prorenin with the enzyme kinetic assay, most investigators add trypsin to their samples to convert prorenin to renin. Unfortunately this is a delicate procedure, because the concentration of trypsin, the duration of the incubation, and the temperature are all critical, and optimal conditions may differ for different specimens *[13]*. Plasma contains serine protease inhibitors; in some plasmas, therefore, inhibition of trypsin may lead to incomplete conversion of prorenin into renin. This may help explain why the plasma prorenin concentrations calculated from the IRMA results (after activation with Remikiren) were somewhat higher than the concentrations calculated from the results of the enzyme kinetic PRC assay (after activation with trypsin). Moreover, in other biological fluids with lower antitrypsin activity, the optimal conditions for the activation of prorenin with trypsin may differ from those in plasma. The higher prorenin results obtained in follicular fluid with the Remikiren-enhanced IRMA, compared to the enzyme kinetic assay results obtained after activation with trypsin, may be related to low antitrypsin activity, which allows prorenin and renin to be exposed to higher effective trypsin concentrations, with an increased risk of prorenin and renin destruction. These problems are not encountered during Remikiren-treatment of the samples.

The interassay CV of the Remikiren-enhanced IRMA, ~8%, was lower than with the enzyme kinetic PRC assay after treatment with trypsin. Our results in plasmas with a low prorenin content indicate that the functional sensitivity of the IRMA is sufficient to identify subjects with low plasma prorenin, e.g., patients with Conn syndrome and some patients with essential hypertension. In fact, prorenin measurements can help to identify hypertensive patients as having so-called low-renin hypertension, because a low renin concentration in these cases is associated with a low concentration of prorenin.

Opinions differ as to whether PRA or PRC is a better measure of the in vivo activity of the renin–angiotensin system *[37]*. As stated above, results of the enzyme kinetic PRC assay are largely independent of the angiotensinogen concentration. In contrast, results of the enzyme kinetic PRA assay are determined by the plasma concentrations of both renin and angiotensinogen. According to some investigators, PRA is a more accurate measure of the in vivo Ang I production than PRC, because the angiotensinogen concentration in plasma is usually within the range where it does influence Ang I production. The rate of Ang I generation for a given concentration of renin in the PRA assay, during incubation of plasma at 37 °C, does indeed differ among different patient groups *[8, 38, 39]*. On the other hand, some evidence indicates that part of the Ang I in plasma is formed in the tissues *[31]*, such that investigators do not know how the production of Ang I in tissue is influenced by the concentration of angiotensinogen in the circulation. Because the IRMA of renin is not influenced by angiotensinogen concentrations, the IRMA-measured PRC values should theoretically be a

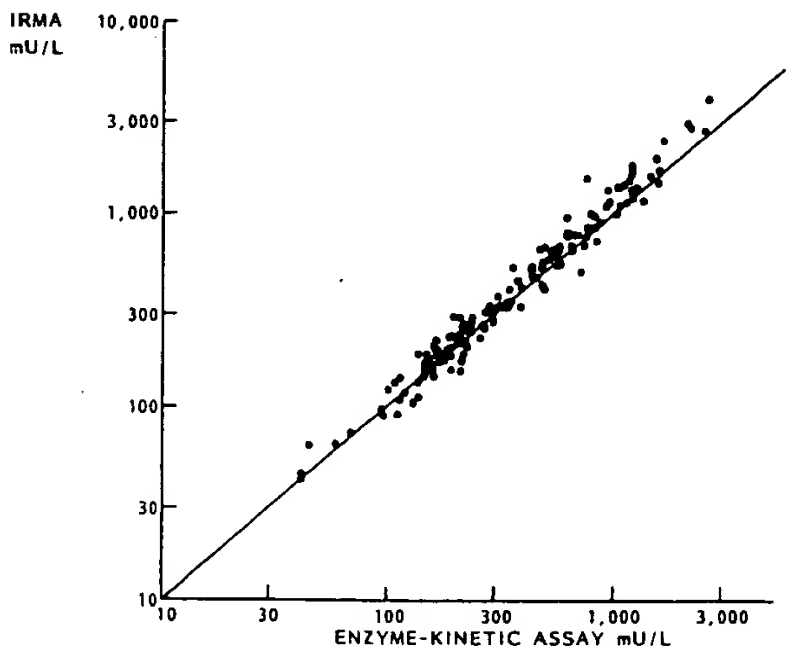

Fig. 7. Comparison between the plasma concentrations of prorenin calculated from the results of the IRMA and of the enzyme kinetic PRC assay.

The least-squares regression equation was obtained after logarithmic transformation of the results (n = 172). The *y*-intercept is given as the antilog. Slope = 0.94, intercept = 1.4, r^2 = 0.98 (P <0.0001). The line of identity is shown.

Table 6. IRMA 0.95 reference limits and geometric means for renin and calculated prorenin in plasma from supine normal subjects.

	Renin	Calculated prorenin
Reference group	mU/L	
All subjects (n = 100)		
Geometric mean	22.0	199
Lower limit	7.7 (6.4–9.0)[a]	88.1 (76.0–95.3)
Upper limit	54.8 (48.9–61.1)	390 (367–412)
Men (n = 50)		
Geometric mean	26.7	223
Lower limit	10.7	109
Upper limit	59.8	405
Women (n = 50)		
Geometric mean	18.1[b]	178[c]
Lower limit	6.5	77.4
Upper limit	46.1	383

[a] The 0.90 confidence limits for the lower and upper level are given in parentheses. Data were analyzed with the parametric method of Solberg *[33]*, after logarithmic transformation.

[b,c] Significantly different from results for men: [b] P <0.01, [c] P <0.05.

more accurate measure of the release of renin from the kidneys than is PRA, particularly in clinical conditions with widely different angiotensinogen concentrations.

We conclude that the new IRMA described here has important advantages over classical enzyme kinetic assays, and that the IRMA is suitable for differentiating between primary and secondary aldosteronism; for diagnosing renovascular hypertension; for detecting high-, medium-, and low-renin essential hypertension; and for monitoring ovarian response to gonadotropin stimulation.

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